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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

AD-A209 971

1b. RESTRICTIVE MARKINGS: NA	
3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Uniformed Services University of the Health Sciences	5. MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a. NAME OF PERFORMING ORGANIZATION: Uniformed Services Univ.	6b. OFFICE SYMBOL (If applicable): NA
7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code): Laser Biophysics Center, USUHS, 4301 Jones Bridge Road, Behtesda, Maryland 20814-4799	7b. ADDRESS (City, State, and ZIP Code): 800 N. Quincy St., Arlington, VA 22217-5000
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research	8b. OFFICE SYMBOL (If applicable): ONR
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-WR-24020	
8c. ADDRESS (City, State, and ZIP Code): 800 N. Quincy St., Arlington, VA 22217-5000	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
61153N	RR04106	4415-806	

11. TITLE (Include Security Classification)

(u) Protein Dynamics Studied with Resonantly Enhanced Quasi-Elastic Light Scattering

12. PERSONAL AUTHOR(S): Reinisch, Lou

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 7/88 TO 7/89	15 June, 1989	9

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Protein Dynamics, Microbalance, Purple Membrane, Light Scattering, Hydration Shell, Resonance Enhancement, Thermal Diffusion

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In order to investigate and understand the dynamics of protein fluctuations, specifically the interaction between dynamical structure and function, we have started a program to used resonantly enhanced light scattering to probe the fluctuation spectrum. Here we report on a new technique, the use of a quartz microbalance to confirm the existance of motion within myoglobin at 5 MHz. We report on modelling the temperature rise for an absorbing sample with incident laser light. Finally, we give some preliminary results of quasi-elastic light scattering on samples of membrane fragments containing the bacteria Bacteriorhodopsin.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(u)

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
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DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE (u)

Annual Report on Contract N00014-WR-24020

R&T Code: 4415806

Date: 1 July, 1989

Principal Investigator: Lou Reinisch

Contractor: Uniformed Services University

Contract Title: Protein Dynamics Studied with Resonantly Enhanced Quasi-Elastic Light Scattering

Research Abstract: The idea that the structure of the protein is important for its function (lock and key model) must be revised: The *dynamical structure* of a protein is important for its function. Consider the protein myoglobin (Mb); it has been used in a wide range of studies. Protein motion in Mb is essential for ligand binding. Investigations have indicated the importance of protein motion, but none have really measured the frequency spectrum of the fluctuations. Recently, flash photolysis and the Mössbauer effect have put limits on the frequency spectrum, but the shape of the spectrum is still unknown. In the work proposed here, we plan to measure the frequency spectrum of protein motion in Mb using Quasi-Elastic Light Scattering (QELS). We can then study the protein fluctuations as a function of the environment, substrate binding and other perturbations such as low temperature and high pressure. QELS has been used extensively to measure protein diffusion coefficients. Unique to our studies, however, will be the employment of a method common to Raman spectroscopy, resonance enhancement. The incident laser light will be in resonance with the chromophore (e.g., heme group) absorption. We can thereby probe parts of the protein and separate structure fluctuations from protein diffusion. The results from this study will be twofold. First, the relationship between the dynamics of the protein and the function of the system can be better understood. In addition, the dynamical structure of the protein is being probed. The outer shell of Mb has been modelled as a semifluid. We would then expect to see correlations between the measured spectrum and the normal modes of oscillations in viscous liquid drops.

Measurement of Protein Hydration Shells Using a Quartz Micro-Balance

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Abstract

The protein myoglobin is dried on the surface electrodes of a 5 MHz quartz crystal oscillator. As the humidity above the film increases, the protein absorbs water and develops a hydration shell. The oscillator frequency shift measures the mass of absorbed water. Dramatic incremental decreases in the amplitude of vibration are also observed, corresponding to a *softening* of the protein structure and subsequent energy dissipation (damping) of the oscillations. The transition of a protein from a dry more rigid structure to a *semifluid* hydrated state is observed to occur in discrete steps.

This first study is presented to demonstrate the viability of the technique, and the important information one can obtain with the quartz microbalance. The experiment can be repeated as a function of crystal frequency, using a number of crystals from 10 kHz to 100 MHz. The damping of the crystal should, of course, decrease when the frequency of the crystal differs from the frequencies of the protein fluctuations. In addition, the temperature can be varied. The temperature of protein will change the spectrum of protein fluctuations. New information concerning the temperature dependence of the protein internal viscosity could be obtained from this technique. The experiment can also be repeated using different proteins. We anticipate a number of interesting and informative studies to follow.

This work has been submitted to Physical Review Letters (1989).

Laser Induced Heating and Thermal Propagation, A Model of Tissue Interaction with Light

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Abstract

The temperature distribution in space and time is modeled for an absorbing tissue with incident laser light. We use *Beer's Law* with absorption coefficient α to describe the light absorbed within the tissue. Heat is diffused away in all directions by the thermal conductivity, κ, of the tissue, and the rate of heat flow is proportional to the temperature gradient. The specific heat, c_p, and the density, ρ, are the remaining parameters in the model. We give the resulting heat flow equation with a source term as the inhomogeneous partial differential equation:

$$\kappa \nabla^2 \mathrm{T} - (c_p \rho) \frac{\partial \mathrm{T}}{\partial t} = -I_0 \alpha \qquad (1)$$

where I_0 is the incident laser intensity. Equation (1) is solved with a *Green's function*. The resulting function:

$$\mathrm{T}(\mathbf{r},t) = \frac{I_0 \alpha \sqrt{c_p \rho}}{16(\pi\kappa)^{3/2}} \int_0^t dt' \int d^3x' \frac{\Psi(\mathbf{r}', t')}{(t-t')^{3/2}} exp\left[\frac{-|\mathbf{r}-\mathbf{r}'|^2 (c_p \rho)}{4\kappa(t-t')}\right] \qquad (2)$$

of the change in temperature for the tissue is numerically integrated. An arbitrary intensity distribution for the incident laser intensity can be chose using a density distribution $\Psi(\mathbf{r}', t')$. In one example calculation, we use a gaussian intensity distribution with an f/5.7 focussed beam to obtain the temperature distribution in cell membranes during a micro-beam experiment. In the micro-beam experiment, a laser is focussed with a microscope and "aimed" at a cell membrane. A micron size hole is subsequently "burned" through the membrane. We assume that the hole is created whenever the absorbed laser light raises the temperature of the water to its boiling point. The values from water are used for density, thermal conductivity and specific heat in our calculation. The absorption coefficient of the cells is measured with a spectrophotometer. Correlations between the volumes where the calculated temperature exceeds the boiling point of water and the measured hole size from micro-beam experiments are compared.

This work is used to characterize the temperature changes in the resonantly absorbing samples during the light scattering experiments. The resonant light can induce thermal effects (*i.e.*, an increase in diffusion rates from a higher temperature and a decrease in solvent viscosity), as well as stimulating the molecular fluctuations. It is, therefore, important to know the temperature in the sample under the exact experimental conditions. Since our calculations agree well with the "calibration" of the microbeam technique–a hole is created when the water reaches boiling–we expect it to correctly describe the temperature profile of a scattering solution.

This was reported at the Conference on Lasers and Electro-Optics in Baltimore, April 1989. A written manuscript is in preparation.

Photo-Induced Membrane Fluctuations from Purple Membrane Detected with Homodyne Quasi-elastic Light Scattering

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Abstract

Membrane fragments containing the protein Bacteriorhodospin are known to pump protons after absorbing light. The photocycle progresses through several intermediate states, as characterized by the absorption spectra, after the absorption of a photon. During this cycle the protein is also known to undergo several conformational transitions in addition to pumping a proton. With low intensity light, the homodyne autocorrelation function shows the membrane fragments to diffuse with rates that correlate well with the size distribution of the membrane fragments. However, when the light is increased, the autocorrelation function shows a dramatic increase the frequency spectrum. Initial measurements have shown a gaussian shaped band of fluctuation frequencies, centered near 1 kHz to be stimulated by the increased light. Thermal effects are currently being checked to explain these additional fluctuations. In addition, the fluctuations are the greatest on the large membrane fragments–those with a diameter greater than 2 μm.

The idea to use quasi-elastic light scattering to measure fluctuations within biomolecules has received little attention. The changes in the autocorrelation spectrum are generally believed to be very small. Using a combination of carefully controlled experimental techniques, computer assisted data analysis and sample preparation, we believe that we have been successful in measuring changes in the scattered light autocorrelation function. We currently interpret the additional motion as a ruffling of the membrane fragment during the photocycle. Work is continuing to identify this motion.

The light scattering experiments using an autocorrelator were started on the purple membrane fragments instead of heme proteins because the larger membrane fragments scatter light more effectively and the small protein molecules in solution. The purple membrane can also resonantly scatter light and one would expect them to exhibit biomolecular fluctuations. Thus the purple membrane is an excellent molecule to study, in addition to a "training molecule." A manuscript is in preparation and the experiments continue.

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